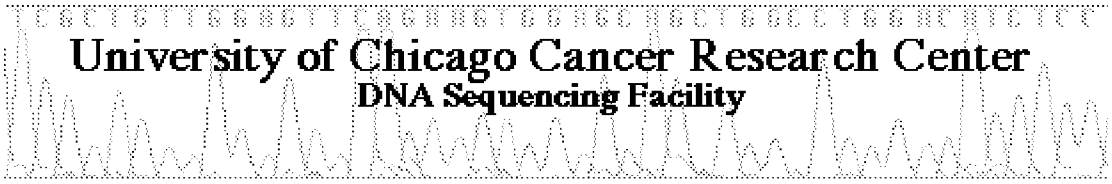

Last updated on March 8, 2007

The Standard Primers

We supply the following standard primers:

- M13for 5' GTAAAACGACGGCCAGT 3'
- M13rev 5' CACACAGGAAACAGCTATGACCAT 3'
- T7long 5' GTAATACGACTCACTATAGGGC 3'
- T7short 5' TAATACGACTCACTATAGGG 3'
- T7term 5' GCTAGTTATTGCTCAGCGG 3'
- T3 5' AATTAACCCTCACTAAAGGGA 3'
- SP6 5' ATTTAGGTGACACTATAG 3'
- SP6Long 5' ATTTAGGTGACACTATAGAATAC 3'
- BGHrev 5' TAGAAGGCACAGTCGAGG 3'
- pGEXFor 5' CTGGCAAGCCACGTTTGGTG 3'
- pGEXRev 5' GGAGCTGCATGTGTCAGAGG 3'
- polyT-G 5' TTTTTTTTTTTTTTTTTTTTTTTTG 3'
- polyT-C 5' TTTTTTTTTTTTTTTTTTTTTTTTC 3'
- polyT-A 5' TTTTTTTTTTTTTTTTTTTTTTTTA 3'
- polyT(ACG) 5' TTTTTTTTTTTTTTTTTTTTTTTTA/C/G 3'
- polyA-G 5' AAAAAAAAAAAAAAAAAAAAAAAAG 3'
- polyA-C 5' AAAAAAAAAAAAAAAAAAAAAAAAC 3'
- polyA-T 5' AAAAAAAAAAAAAAAAAAAAAAAAT 3'
- polyA(CGT) 5' AAAAAAAAAAAAAAAAAAAAAAAAC/G/T 3'

For large templates such as BAC's, PAC's and cosmids, we recommend the use of the SP6long primer instead of the standard SP6 primer, which has a marginally low Tm. The SP6long primer is four bases longer (so check for compatibility with your vectors) but works well for large templates when the shorter SP6 primer fails.

Primer Design Considerations

One of the single most important factors in successful automated DNA sequencing is proper primer design. It is important that a primer has the following characteristics:

- A melting temperature (Tm) in the range of 50 C to 65 C
- Absence of dimerization capability
- Absence of significant hairpin formation (>3 bp)
- Lack of secondary priming sites
- Low specific binding at the 3' end (ie. lower GC content to avoid mispriming)

Primers designed according to these criteria will generally be from 18 to 30 bases in length and have %

GC of 40 to 60. Try to avoid using primers with Tm's above 65-70 C, especially on high GC templates, as this can lead to secondary priming artifacts and noisy sequences. We strongly recommend the use of computer software to design primers with these characteristics. Examples of such software are: LaserGene (DNAStar), Oligo (National Biosciences, Inc.), MacVector (Kodak/IBI) and the GCG suite. In addition, there is a web site available for designing PCR primers using the Primer program (Whitehead Institute). In lieu of software, the following equation can be used to roughly estimate Tm:

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Tm = 59.9 + 0.41*(%GC) - 600/length
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If designing a primer based on existing sequencing data, choose a priming site that is greater than 50 nucleotides away from the position where new sequence is needed. Avoid designing primers using regions of poorer quality sequence, such as areas beyond single peak resolution of a chromatogram (typically 600-700 bases). Avoid primers where alternative priming sites are present with more than 90% identity to the primary site or that match at more than seven consecutive nucleotides at the 3' end.

Finally, be aware that no set of guidelines will always accurately predict the success of a primer. Some primers may fail for no apparent reason, and primers that appear to be poor candidates may work well.